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(54) Title: DERIVATION OF HUMAN TUMOR CELL LINES USING PASSAGE IN TRANSGENIC MICE HAVING SELECTABLE MARKERS

(57) Abstract: The invention a method for isolating substantially purified cells and cell lines. Cells or tissue (for example, tumor tissue) from a donor animal is xenografted into a recipient transgenic immunodeficient animal which has been genetically engineered to exhibit a selectable trait. Upon removal of the donor cells from the recipient animal, the cells are placed in culture under conditions that allow contaminating recipient cells to be distinguished from donor cells by means of the selectable trait. This allows the removal of the contaminating recipient cells, resulting in a substantially pure culture of donor cells. A population of substantially pure cells, cell lines and vaccines made from such cells, and a method of testing compounds using such cells, are also provided.

DERIVATION OF HUMAN TUMOR CELL LINES USING PASSAGE IN TRANSGENIC MICE HAVING SELECTABLE MARKERS

DESCRIPTION

BACKGROUND OF THE INVENTION

Field of the Invention

The invention generally relates to the isolation of purified cells and cell lines. In particular, the invention provides a method for isolating purified cells by passaging cells or tissue from a donor animal in a recipient transgenic immunodeficient animal, wherein the transgenic animal has been genetically engineered to exhibit a selectable trait.

Background of the Invention

The isolation of pure cultures of cells is highly desirable for many reasons. Purified tumor cells can be used to establish tumor cell lines for investigative purposes, such as the *in vitro* testing of drug candidates, or for purposes of research directed to the characterization and understanding of tumor cells. Yet another desideratum is the development of patient-specific tumor vaccines which would be raised against purified tumor cells isolated from the patient and grown in culture.

Unfortunately, there are many difficulties that attend the isolation of human tumor cell lines, especially those from solid tumors such as sarcomas and carcinomas. Human tumors are known to be composed of two major categories of cells, the neoplastic cells that drive the formation of the tumor and that account for its morbidity and mortality, and the non-neoplastic host cells. The latter include the parenchymal cells of an organ that has been invaded by the tumor, stromal cells such as endothelial and fibroblast cells that form the supportive stroma of the tumor and whose presence may be stimulated by tumor products, inflammatory cells that react to tissue damage and other local alterations of the tumor site, and other categories of cells. A major technical obstacle is that in the first days or weeks of attempts to culture human tumor cells from isolates of the tumor, the cancer cells proliferate more slowly than do some of the non-neoplastic elements. Most cultures therefore become

overrun by non-neoplastic cells having a primitive fibroblast-like growth pattern.

A method to eliminate the human non-neoplastic cells would greatly aid the isolation of new human tumor cell lines. Methods attempted in the past include the use of special coatings on the tissue culture surfaces to select for epithelial rather than stromal cell adherence; such methods offer some improvement in individual cases but are not sufficiently efficient to serve as a general solution to the problem.

Another method to eliminate the non-neoplastic cells is the passage of a human tumor as a xenograft in another animal, usually an immumodeficient mouse strain such as the nu/nu mouse or the SCID mouse. The human tumor cells become supported by mouse tissues, and the human neoplastic cells proliferate while the non-neoplastic human cells do not grow and may even die. In human pancreatic cancer, for example, 90% of all human tumors can be successfully passaged as xenografts in nu/nu or SCM mice (1). However, attempts to culture cells from the xenografted tumors are hampered by a problem analogous to that encountered during the direct isolation of tumor cells from humans: contaminating mouse cells tend to overgrow the culture and hinder the isolation of pure human tumor cells.

It would be highly desirable to have available a method to isolate and culture purified human tumor cells. The present invention provides a new method whereby mouse cells can be selectively eliminated from such cultures using a mouse of a special Type, one having innate selectable biological characteristics.

SUMMARY OF THE INVENTION

It is an object of this invention to provide a method for obtaining a substantially purified population of cells. The method involves the steps of obtaining donor cells from a donor organism and implanting the donor cells into a non-human transgenic immunodeficient recipient organism. The cells of the transgenic immunodeficient recipient organism exhibit a selectable trait. The donor cells are harvested from the transgenic immunodeficient recipient organism, placed in culture, and the contaminating transgenic immunodeficient recipient cells are selected via the selectable trait. The selected contaminating transgenic immunodeficient recipient cells may then be removed from the culture, thus providing a substantially purified population of cells. In a preferred

embodiment of the invention, the transgenic immunodeficient recipient organism is a mouse.

The donor cells may be, for example, tumor cells, pancreatic islet cells, lymphocytes or hematopoietic bone marrow cells. The selectable trait may be, for example, the ability to express a toxic protein (for example, thymidine kinase), the ability to express a detectable marker, a biochemical deficiency, or sensitivity to a selection agent. In a further aspect, the method may also include the step of activating an inducible promoter that controls transcription of a gene encoding the selectable trait. Examples of such promoters include the tetracycline-inducible promoter, the lac-inducible promoter, and the metallothionein promoter.

In the practice of the method, the step of selecting may be carried out by exposing the cultured cells to an effector such as a selection agent (e.g. gangcyclovir) or an environmental stimulus such as light, a temperature change, or withdrawal of an essential nutrient

In the practice of the method, the step of removing may be carried out by mechanical removal of the contaminating transgenic immunodeficient recipient cells, or by inducing the death of the contaminating transgenic immunodeficient recipient cells.

In yet another aspect of the method, cell lines may be established from the substantially purified population of cells produced by the method.

It is a further object of the instant invention to provide a substantially purified population of cells which have been purified by the steps of: obtaining donor cells from a donor organism and implanting the donor cells into a non-human transgenic immunodeficient recipient organism. The cells of the transgenic immunodeficient recipient organism exhibit a selectable trait. The donor cells are harvested from the transgenic immunodeficient recipient organism, placed in culture, and the contaminating transgenic immunodeficient recipient cells are selected via the selectable trait. The selected contaminating transgenic immunodeficient recipient cells may then be removed from the culture, thus providing a substantially purified population of cells. Cells of this type include tumor cells, pancreatic islet cells, lymphocytes and hematopoietic bone marrow cells.

It is a further object of the instant invention to provide a vaccine comprising a substantially purified population of cells which have been purified by the steps of: obtaining donor cells from a donor organism and implanting the donor cells into a non-human

transgenic immunodeficient recipient organism. The cells of the transgenic immunodeficient recipient organism exhibit a selectable trait. The donor cells are harvested from the transgenic immunodeficient recipient organism, placed in culture, and the contaminating transgenic immunodeficient recipient cells are selected via the selectable trait. The selected contaminating transgenic immunodeficient recipient cells may then be removed from the culture, thus providing a substantially purified population of cells. The vaccine further comprises a physiological acceptable carrier, and may comprise an adjuvant.

It is a further object of the instant invention to provide a non-human, immunodeficient animal (such as a mouse) in which the animal's cells are transgenic for a selectable trait. The mouse may be, for example, the offspring of a nu/nu mouse, e.g., a tox/tox:nu/nu mouse. The selectable trait exhibited by the animal may be, for example, the ability to express a toxic protein (such as thymidine kinase), the ability to express a detectable marker (such as green fluorescent protein, luciferase, expression of a cell surface protein or expression of a cell surface polysaccharide), a biochemical deficiency (such as ARIFT deficiency or HPRT deficiency), or sensitivity to a selection agent.

It is a further object of the instant invention to provide a method for testing the effect of a compound on a substantially purified population of cells of a selected type. The method involves procuring a population of substantially purified cells of the selected type, exposing the cells to the compound, and observing the effect of the compound on the cells. The population of cells is procured by obtaining donor cells from a donor organism and implanting the donor cells into a non-human transgenic immunodeficient recipient organism, the cells of which exhibit a selectable trait. The implanted cells are harvested, placed in culture, and contaminating transgenic immunodeficient recipient cells in the culture are selected via the selectable trait. Selected contaminating cells are then removed from the culture. Compounds that may be tested in this manner include drugs, toxins, anti-tumor agents, libraries of chemicals, extracts, peptides, and polysaccharides.

It is a further object of the instant invention to provide a cell line, comprised of a substantially purified population of cells. The population of cells is procured by obtaining donor cells from a donor organism and implanting the donor cells into a non-human transgenic immunodeficient recipient organism, the cells of which exhibit a selectable trait.

The implanted cells are harvested, placed in culture, and contaminating transgenic immunodeficient recipient cells in the culture are selected via the selectable trait. Selected contaminating cells are then removed from the culture. The cell line may be immortal, and in a preferred embodiment, the cells are tumor cells.

It is a further object of the instant invention to provide a test kit for the testing or evaluating of properties of compounds which may have an effect on a substantially purified population of cells. The kit comprises a substantially purified population of cells, medium, and a container to contain the cells and medium. The population of cells is procured by obtaining donor cells from a donor organism and implanting the donor cells into a non-human transgenic immunodeficient recipient organism, the cells of which exhibit a selectable trait. The implanted cells are harvested, placed in culture, and contaminating transgenic immunodeficient recipient cells in the culture are selected via the selectable trait. Selected contaminating cells are then removed from the culture.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic representation of the method of obtaining purified tumor cells. 10, tumor mass; 11, transgenic mouse; 12. xenografted tissue from tumor; 13, container for harvested cells; 14, disaggregated tumor cells; 15, contaminating transgenic cells; 16, contaminating transgenic cells exhibiting selectable trait.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The present invention provides a method for obtaining substantially purified populations of cells. The method involves the transplantation (xenografting) of cells or tissue from a donor animal into a transgenic recipient organism or animal. The cells of the transgenic recipient have been genetically altered to exhibit a selectable trait. Upon removal from the transgenic recipient animal, the donor cells or tissue are cultured *in vitro*. The culture is exposed to an agent or to permissive conditions that permit expression and

detection of the selectable trait of any transgenic donor cells which are present in the culture. It is thus possible to distinguish donor cells from recipient cells, and ultimately to remove the latter from the culture, resulting in a substantially purified culture of donor cells.

By "substantially purified" we mean that within the population of cells, greater than about 90% of the cells in the population of cells are of a single, desired type. Examples of desired types of cells that can be substantially purified by the method of the instant invention include but are not limited to tumor cells, pancreatic islet cells, lymphocytes, and hematopoietic bone marrow cells.

In one embodiment of the present invention, the donor cells are tumor cells. In this case, excised tumor material from a patient is passaged in a transgenic animal. The cells of the transgenic animal have been genetically modified to contain a selectable trait. Following removal of the xenografted tumor material from the transgenic animal, the tumor material is cultured *in vitro* and is exposed to a selection agent or environmental condition that allows the selectable trait to be expressed. Expression of the selectable trait allows the tumor cells to be distinguished from and thus separated from the cells of the transgenic animal.

According to the method of the present invention, the cells of the transgenic recipient animal exhibit a selectable trait. By "selectable trait" we mean a trait that, when exhibited, allows the cells of the transgenic recipient animal to be distinguished from and separated from the donor cells. Those of skill in the art will recognize that many selectable traits exist which can be genetically engineered into the recipient animal cells. Examples include but are not limited to sensitivity to a selection agent, ability to express a toxic protein, inability to express a protective or otherwise necessary protein, ability to express a detectable marker, possession of a biochemical deficiency, and the like.

For example, upon exposure of the harvested, donor cell culture to a selection agent, the transgenic animal cells in the culture may grow more slowly or cease to grow, allowing the tumor cells of interest to flourish, thereby greatly facilitating their isolation.

In one embodiment of the instant invention, the cells of the transgenic recipient animal are genetically engineered to contain a gene that causes the cells to exhibit sensitivity to a selection agent. In this case, exposure of the transgenic cells to the selection agent causes a deleterious effect in the transgenic cells. For example, the transgenic cells may grow more slowly, cease to grow altogether, or the effect may be toxic, i.e. they may be killed.

Those of skill in the art will recognize that many examples of such genes and their corresponding selection agents exist which can be employed in the practice of the present invention. Examples of suitable genes include but are not limited to viral thymidine kinase.

The gene which confers the sensitivity to a selection agent may be constituitive (i.e. the sensitivity trait is expressed at all times, however the effect of the selection agent is not elicited until the cells are exposed to the selection agent in culture). Alternatively, the gene may be inducible, i.e. induction of sensitivity may occur by way of, for example, an inducible promoter. In this case, the sensitivity trait is not expressed until the promoter is induced (e.g. in culture after harvesting of the xenografted cells/tissue). This necessitates the addition of an inducing agent to the cultured cells. Following addition of the inducing agent, the selectable trait is expressed by the non-neoplastic transgenic cells so that, upon exposure to the selection agent, the desired effect is elicited. Those of skill in the art will recognize that many suitable inducible promoters exist that can be utilized in the practice of the present invention, including but not limited to the tetracycline-inducible promoter, the lac-inducible promoter (which may be activated by the allo-lactose analog IPTG), and the metallothionein promoter (activated by heavy metals zinc and cadmium).

In some embodiments of the present invention, the selectable trait is such that a desired effect is obtained by exposing the cultured, xenografted donor cells to a selection agent. Those of skill in the art will recognize that many suitable selection agents exist which are suitable for use in the present invention when their use is coupled with a corresponding selectable trait. Examples of such selection agents include but are not limited to gancyclovir (when the recipient animal is transgenic for the thymidine kinase gene).

Alternatively, the selectable trait may be one that is elicited without exposure to a selection agent per se, rather the trait is expressed (either constituitively or inducibly) and the corresponding effect is either readily obvious (e.g. the cells are a specific color, shape, or have some otherwise distinguishing characterisitc) or the effect can be elicited by an environmental stimulus. For example, the transgenic cells may be temperature sensitive in nature such that, so long as the cells are within the transgenic animal itself, they remain viable, but upon exposure to an elevated temperature in culture, they are rendered non-viable. Any suitable selectable trait, the effects of which are elicited by an environmental stimulus, may be used in the practice of the present invention.

Alternatively, the cells of the transgenic recipient animal may display a selectable trait such as a biochemical deficiency. The biochemical deficiency may be the result of genetic engineering, or the result of selective breeding. In this case, the transgenic animal may be maintained by providing the animal with supplements that counter the deficiency, or the animal tissue may partially tolerate the deficiency, but once the xenografted tumor is harvested and placed in culture, the supplement is withdrawn, or the culture conditions are altered to exaggerate the detrimental effects of the deficiency. In such culture conditions, the non-neoplastic transgenic cells will die and thus be eliminated from the culture. Examples of such biochemical deficiencies include but are not limited to ARIFT (adenine phosphoribosyl transferase) deficiency, and HPRT (hypoxanthine phosphoribosyl transferase) deficiency. APRT-null and HPRT-null cells will die when grown in special purine-deficient media such as HAT media.

Alternatively, the selectable trait may a detectable marker which confers upon the cells of the transgenic animal a property that allows the cells of the transgenic animal to be readily distinguished from the donor cells without having a deleterious effect on the contaminating transgenic animal cells. In such a case, the contaminating transgenic animal cells may be identified and physically removed or separated from the desired donor cells. Such detectable markers may be constituitive (so long as they do not compromise viability of the transgenic recipient animal) or inducible in that they are not expressed in the transgenic animal but are manifested in the cultured cells of the harvested xenograft. Manifestation of the marker may be by adding an agent to the culture that allows either induces expression of the selectable trait, or that facilitates detection of the selectable trait, or a combination of both. For example, the marker may be expressed upon induction of an inducible promoter encoding a protein or proteins responsible for the selectable trait. The marker may then be directly detectable, or may be detected by the addition of an agent that permits such detection. Alternatively, the detectable marker may be manifested in response to an environmental stimulus such as a certain wavelength of light, a change of temperature, etc.

Those of skill in the art will recognize that many such suitable detectable markers exist which can be utilized in the practice of the present invention. Examples of suitable detectable markers include but are not limited to expression of jellyfish green fluorescent

protein (and the many variations thereof), expression of luciferase (and variants thereof), expression of a distinguishing cell surface protein or polysaccharide that allows affinity capture of the transgenic cells, and the like. In this case, the ability to clearly differentiate between donor cells and transgenic animal cells in culture allows the transgenic animal cells to be mechanically removed from donor cells by any of a variety of techniques which are well known to those of skill in the art, for example, cell sorting, clonal islolation, solid state affinity capture (e.g. affinity chromatography), and the like.

Those of skill in the art will recognize that a wide variety of cells may be amenable to purification by the methods of the present invention. Examples of suitable cell types include but are not limited to tumor cells, pancreatic islet cells, lymphocytes and hematopoietic bone marrow cells. Further, the cells which are purified by the methods of the present invention may originate from any species (including humans), so long as it is desirable to obtain purified cells from that species. In addition, the recipient transgenic animal may be from any non-human species as well, so long as the recipient animal is transgenic for an appropriate selectable trait, and is capable of receiving and sustaining the xenografted cells or tissue material for an appropriate period of time.

In a preferred embodiment of the present invention, the cells which are purified by the methods of the instant invention are tumor cells. Such a purification is illustrated in Figure 1. Briefly, a suitable portion 12 of a tumor mass 10 is implanted (xenografted) into a transgenic mouse 11. After a period of time, the portion of the tumor mass 12 is harvested, disaggregated, and cells (which will include tumor cells 14 and contaminating transgenic cells 15) are placed in culture in an appropriate container 13. Non-tumor cells are selected, and selected non-tumor cells 16 are removed from the culture. A population of substantially purified tumor cells 15 are left behind. Those of skill in the art will recognize that many types of tumor cells exist which are amenable to purification according to the methods of the present invention. Examples include but are not limited to colorectal cancer, pancreatic cancer, leukemia and lymphoma.

In the practice of the present invention, a transgenic recipient animal is utilized to receive implanted donor cells or tissue. In a preferred embodiment of the present invention, the transgenic recipient animal is a mouse. There are a number of methods to introduce the exogenous DNA into the germ line of an animal. One method is by microinjection of the

gene construct into the pronucleus of an early stage embryo (e.g., before the four-cell stage) (Wagner, et al., 1981; Brinster, et al., 1985). The detailed procedure to produce such transgenic mice has been described (see e.g., Hogan, et al., 1986; U.S. Pat. No. 5,175,383, 1992). This procedure has also been adapted for other mammalian species (e.g., Hammer, et al., 1985; Murray, et al., 1989; Pursel, et al., 1987; Rexroad, et al., 1990; Rexroad, et al.,1989; Simons, et al.,1988; Vize, et al., 1988; and Wagner, J.1989).

Another method for producing germ-line transgenic mammals is through the use of embryonic stem cells. The gene construct may be introduced into embryonic stem cells by homologous recombination (Thomas, et al., 1987; Capecchi, 1989; Joyner, et al., 1989) in a transcriptionally active region of the genome. A suitable construct may also be introduced into the embryonic stem cells by DNA-mediated transfection, such as electroporation (Ausubel, et al., 1987). Detailed procedures for culturing embryonic stem cells and the methods of making transgenic mammals from embryonic stem cells can be found in Teratocarcinomas and Embryonic Stem Cells, A practical Approach, ed. E. J. Robertson (IRL Press, 1987).

In the above methods for the generation of germ-line transgenic mammals, the construct may be introduced as a linear construct, as a circular plasmid, or as a viral vector which may be incorporated and inherited as a transgene integrated into the host genome. The transgene may also be constructed so as to permit it to be inherited as an extrachromosomal plasmid (Gassmann, M. et al., 1995). The term plasmid is meant to describe a DNA molecule that can replicate autonomously in a host such as a bacterium or yeast.

In a preferred embodiment of the instant invention, the DNA construct contains a gene encoding a selectable trait ("st"), which may be under the control of an inducible promoter. Embryonal cells having stably integrated the gene into their chromosomes can be developed into a strain of homozygous animal having two copies of the integrated construct in every cell, an st/st animal, or can be maintained in the heterozygous state, as st/- animals. In order to establish founder strains in which all cells of the animal are transgenic, it is necessary to produce animals in which some of the germ cells contain the desired transgene for further breeding. Some offspring of such animals, those offspring that contain the transgene, will have the transgene heterozygously in both the germ cells and in the somatic cells, allowing, respectively, the propagation and the productive use of the strain. This strain

can be bred to homozygosity if desired for the optimal properties of the transgene. The homozygous of heterozygous animal is then cross-bred to a strain of immunodeficient animals (such as the nu/nu mouse), creating animals that contain the selectable trait (either homozygous or heterozygous, as desired) and that are also homozygous for immunodeficiency (for example, a st/st:nu/nu or st/-:nu/nu mouse).

Confirmation of the presence of the desired gene(s) in the chromosome of the founder animals and their offspring may be carried out by genetic analysis of cells of the animals by, for example, Southern hybridization of cellular DNA with a radioactive probe specific for sequences within the gene of interest. Techniques for the analysis of DNA insertions are well known and readily available. See, for example, Maniatis et al., 1982. Animals with the correct genotype may be selected for use in the practice of the present invention.

In a preferred embodiment of the present invention, the transgenic recipient animal is a mouse. Thus, in yet another aspect of the present invention, there is provided a transgenic mouse, the cells of which exhibit or can be induced to exhibit a selectable trait. Those of skill in the art will recognize that many suitable types of mice exist which are appropriate to genetically engineer in order to produce the mice appropriate for use in the practice of the present invention. Examples of such mice include but are not limited to GFP-expressing mice and APRT/HPRT-deficient mice.

In the practice of the method of the present invention, appropriate transgenic recipient mice are implanted with cells or tissue from a donor. In a preferred embodiment of the present invention, the cells or tissue are tumor cells or tissue. Those of skill in the art will recognize that many methods for obtaining cells or tissue from a donor exist and are practiced routinely. With respect to tumors, in general, a small (about 2 mm) fragment of an excised tumor is treated by exposing the fragment to a substance that aids in vascularization (e.g. Matrigel). The exact fragment size is not critical to the practice of the invention and may vary from case to case. The fragment is then implanted subcutaneously into the transgenic recipient, e.g. a st/st:nu/nu or st/-:nu/nu mouse, and allowed to grow. The precise length of time the tumor is allowed to grow and the precise size of the tumor upon harvesting are not critical to the practice of the invention and may vary from case to case. In general, the tumor will be allowed to grow for about one month and will be about 1 cm in diameter when

harvested. In other embodiments, the implanted cells are not tumor cells. For example, human fetal thymus and bone can be xenotransplanted to severe combined immunodeficient (SCID) mice to make a humanized blood cell system in mice (Leukemia 1993; 7 Suppl 2:S98-101) and human blood mononuclearcells can be grafted to SCID mice by intraperitoneal injection of 10 million human cells; these cells persist for over two months (Eur. J. Immunol. 1996; 26:1088-93).

Cells or tissue fragments are harvested using well-known techniques after an appropriate period of time as described above, according to techniques that are well-known to those of skill in the art.

After harvesting, the cells are obtained and, if needed, disaggregated by any of a variety of techniques which are well known to those of skill in the art, e.g. by bleeding, or processing of tissues by mincing, by enzyme digestion, and the like, and placed into a culture medium. Those of skill in the art will recognize that many types of culture medium exist which would be appropriate for use in the practice of the present invention, depending on the type of cells to be cultured. The exact choice of culture medium is not a critical feature of the invention, so long as the desired cultured cells are able to thrive in the medium. Further, the medium may contain various additives which are beneficial for cells in general, or for the specific type of cells to be cultured. Examples include but are not limited to DMEM and RPMI media, and additives include fetal bovine serum, various growth factors, and the like. In addition, the culture medium may contain the selection agent described above for causing the expression of the selectable trait of contaminating transgenic cells, and/or an agent for inducing the gene which encodes a protein responsible for such a selectable trait. Methods for then removing the contaminating cells from the cells of interest were discussed above, and include the mechanical removal via affinity selection techniques, removal via death of the contaminating cells and, for example, decanting the media which contains the dead cells, or removal via a technique which depends on visual recognition of the selectable trait, and the like. Removal of the contaminating cells results in the production of a substantially pure population of donor cells. Thus, in another aspect of the present invention, a substantially purified population of cells is provided, wherein the cells are purified by the methods of the present invention. In preferred embodiments, the substantially purified population of cells may be tumor cells, pancreatic islet cells, lymphocytes or hematopoietic progenitor cells.

In some instances of the practice of the present invention, removal of contaminating transgenic recipient cells may be effected immediately upon harvesting the xenografted cells (i.e. prior to culturing the cells). This may be the case, for example, when the selectable trait is constituative and is detectably expressed in the cells of the transgenic animal. For example, if the selectable trait is the constituative expression of GFP, it may be possible to disaggregate the harvested cells and submit them to a selection procedure (such as cell sorting) prior to culturing the cells. Further observation and sorting may also be carried out during culturing of the cells if necessary to obtain a pure culture.

In another aspect of the instant invention, cell lines may be established from the substantially purified population of cells which is produced by the methods of the present invention. The cell lines may be immortalized, or they may not be immortalized, depending on the application of the cell lines. Cancer cells are already immortal. Lymphocytes can be converted into an immortal culture or immortal line by transformation, for example, using Epstein-Barr virus or by the introduction of viral proteins.

In yet another aspect, the instant invention provides a vaccine comprising cells which are purified by the method of the present invention. The vaccine preparation further comprises a physiologically acceptable carrier, and may include an adjuvant. In a preferred embodiment, the cells which comprise the vaccine are tumor cells, and may most preferably be human tumor cells. For the preparation of such a vaccine, tumor cells or tissue from a tumor is removed from a patient, transplanted into a transgenic recipient animal, harvested from the animal, and placed in culture. The contaminating transgenic recipient animal cells are then identified and removed or separated from the cells of interest (the tumor cells) to yield a purified population of tumor cells as described above. The purified tumor cells are then irradiated to ensure that they will be unable to propagate further and are reinjected into the patient where they act as an autologous tumor vaccine, i.e. they stimulate an immune response. Thus, the immune system of the patient is primed to recognize and destroy other tumor cells (e.g. those which may have metastasized) which exhibit the same antigenic determinants as the purified tumor cells. Procedures for developing vaccines from purified cells are well known to those of skill in the art, as described in Curr Opin Investig Drugs 2001; 2:133-5.

The preparation of such vaccines is well known to those of skill in the art. Typically,

vaccine compositions are prepared either as liquid solutions or suspensions, however solid forms such as tablets, pills, powders and the like are also contemplated. Solid forms suitable for solution in, or suspension in, liquids prior to administration may also be prepared. The preparation may also be emulsified. The active ingredients may be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredients. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol and the like, or combinations thereof. In addition, the composition may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and the like. In addition, the composition may contain other adjuvants. If it is desired to administer an oral form of the composition, various thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders and the like may be added. The composition of the present invention may contain any such additional ingredients so as to provide the composition in a form suitable for administration. The final amount of active ingredient in the formulations may vary. However, in general, the amount of active ingredient (purified cells) in the formulations will be from 1-99%.

The present invention also provides a method for eliciting an immune response in a subject, comprising administering to said subject an effective amount of a vaccine comprising the purified tumor cells of the present invention. By effective amount we mean an amount of the composition of the present invention necessary to prevent, cure or at least partially arrest the growth and metastasis of tumors bearing the same antigenic of the subject. The subject may be any vertebrate, for example, any mammalian (e.g. human) or avian species. The exact amount of the vaccine to be administered will vary from subject to subject and may depend on, for example, weight, gender, age, overall physical condition, and the like. Such variables well understood by those of skill in the art and are best assessed by skilled practitioners in the art.

The vaccine of the present invention may be administered in any of a variety of ways which are well known to those of skill in the art and include but are not limited to: parenterally (e.g. subcutaneously or by intradermal or intramuscular injection), orally, opthalmically, vaginally, rectally, intranasally, transdermally, and the like. Any appropriate method of administration may be utilized in the practice of the present invention so long as the compositions are delivered in an effective manner.

The present invention also encompasses a method for testing compounds. The method involves obtaining a substantially purified population of cells by the methods described herein, and exposing the cells to the compound to be tested. The compound may be any compound of interest, examples of which include but are not limited to drugs, toxins, anti-tumor agents, libraries of chemicals, extracts, peptides, polysaccharides, and the like. In particular, since the methods of the present invention are highly amenable to establishing substantially purified populations of tumor cells, the method would be especially suited to the testing of anti-tumor agents.

To that end, the present invention also encompasses a test kit for the testing or evaluating of properties of compounds which may have an effect on a substantially purified population of cells. The kit comprises substantially purified cells and an appropriate culture medium, and a container for the cells and medium. In a preferred embodiment of the present invention, the kit is a diagnostic kit for testing or assessing the effects of putative anti-tumor compounds and the substantially purified cells of the kit are tumor cells, as illustrated, for example, by the container 13 and substantially purified cells 15 of Figure 1.

The following Examples serve to illustrate various embodiments of the above-described invention but should not be interpreted so as to limit the invention in any way.

EXAMPLES

EXAMPLE 1. Creation of an immunodeficient transgenic mouse with a selectable thymidine kinase gene

A novel transgenic mouse may be constructed by nonhomologous: recombination of an engineered plasmid DNA sequence into mouse embryonal stem cells. The plasmid preferably contains the selectable gene viral thymidine kinase, under the transcriptional control of the tetracycline inducible promoter. Embryonal cells having stably integrated the construct into their chromosomes are selected nucleic acid sequence-specific detections systems and may be used to create a strain of homozygous mice having two copies of this integrated construct in every cell of their body. The construct is not toxic in the absence of tetracycline. The resulting transgenic mouse is designated the tox/tox mouse.

Tox/tox mice may be bred to nu/nu immunodeflcient mice to create mice having two

copies of each genetic trait, termed the tox/tox:nu/nu mouse. These mice are maintained in isolator cages as is standard for immunodeficient mice, and may be used as a transgenic recipient animal.

EXAMPLE 2. Xenografting, culturing and purification of human tumor cells.

Human tumors may be obtained from patients undergoing surgery for therapeutic indications. Small (2mm) fragments of the excised tumors are dipped in Matrigel (Collaborative Research, a basement membrane-like biological substance used routinely as an aid to vascularization of grafts) and implanted subcutaneously into nu/nu mice and into tox/tox;nu/nu mice. After a month, the tumors, which are about 1 cm in size, are harvested utilizing sterile technique. Tumor cells are disaggregated by mincing and are placed into standard culture medium containing DMEM media (Gibco), 20% fetal bovine serum, and added growth factors (the latter reflect a cocktail of growth factors that are recognized as useful for the specific tumor type). Tetracycline (doxycycline, 1μg/ml final concentration) ± the nucleoside analog gangcyclovir (2 μM final concentration) is added to tumor cell cultures from both the nu/nu mice and the tox/tox;nu/nu mice; sterile saline ± gangcyclovir is added to duplicate cultures as a control.

The cell cultures from tumors grown in nu/nu mice show very active cell proliferation and become overrun by confluent sheets of spindle cells resembling fibroblasts by two weeks, irrespective of the addition of tetracycline and/or gangciclovir. These culture conditions are therefore, as expected, deemed inappropriate for the facile isolation of human tumor cell lines.

Cell cultures from tox/tox:nu/nu mice have a similar appearance in the absence of tetracycline or in the absence of gangcyclovir. However, in the presence of both tetracycline and gangciclovir, the tumor cell cultures from tox/tox:nu/nu mice display a significantly different appearance. In these cultures, the numbers of cells with fibroblast morphology are substantially reduced or eliminated, and isolated cells and small colonies of cells with a polygonal, epithelial-like morphology (i.e. tumor cells) are identified. This is because the addition of tetracycline to these cultures results in a high-level of expression of thymidine kinase which is toxic in the presence of otherwise nontoxic amounts of gangciclovir. With time, cultures derived from tox/tox:nu/nu mice progress to confluent sheets characteristic of

carcinoma cells in culture. These cultures can be passaged indefinitely, becoming established human tumors cell lines.

The neoplastic nature of the tumor cell cultures is confirmed by molecular genetic analyses, in which it is confirmed that the oncogene and tumor-suppressor gene mutations of the patient's primary tumors are identical to the mutations of the cell lines produced from these tumors.

EXAMPLE 3. Creation of an immunodeficient transgenic mouse with a detectable green fluorescent protein (GFP) gene

In a second example, transgenic mice that express the detectable green fluorescent protein (GFP) marker (Chalfie et al., 1994) are generated. In this variation, GFP-expressing mouse cells are eliminated from the disaggregated tumor cells or from the cultures by flow cytometric sorting (Bachl et al, 1999). The non-fluorescing cells that are enriched in human tumor calls are retained.

EXAMPLE 4. Creation of an immunodeficient mouse with a selectable biochemical deficiency.

It is also possible to utilize mice that contain homozygous genetic mutations that conveyed a biochemical deficiency such as APRT enzyme defciency (Stockelman et al, 1998) or HPRT deficiency (Edamura and Sasai, 1998). In these cases, special culture media (for APRT-negative mice, medium containing azaserine and adenine; for HFRT-negative mice, HAT medium containing hypoxanthine, ainiaopterin, and thyzddine) eliminates the mouse cells which harbor the metabolic defect while the human tumor cells, which do not have the defect, remain alive in the culture.

EXAMPLE 5. Cancer treatment

Pure populations of their own tumor cells are isolated from an individual cancer patients within a 1-2 month period by use of an enzymatic disaggregation of a harvested xenograft followed by cell sorting. These patient-specific tumor cell expansions are irradiated and reintroduced into the patient as an autologous tumor vaccine. Cultures obtained by the selectable marker technique are tested for anti-cancer drug sensitivities to suggest the optimal chemotherapeutic strategy, individualized for each patient.

While the invention has been described in terms of its preferred embodiments, those skilled in the art will recognize that the invention can be practiced with modification within the spirit and scope of the appended claims. Accordingly, the present invention should not be limited to the embodiments as described above, but should further include all modifications and equivalents thereof within the spirit and scope of the description provided herein.

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The references cited herein are incorporated by reference in their entirety

We claim:

1. A method for obtaining a substantially purified population of cells, comprising the steps of

obtaining donor cells from a donor organism,

implanting said donor cells into a non-human transgenic immunodeficient recipient organism, wherein cells of said transgenic immunodeficient recipient organism exhibit a selectable trait,

harvesting said donor cells from said transgenic immunodeficient recipient organism,

placing said donor cells in culture,

selecting contaminating transgenic immunodeficient recipient cells in said culture via said selectable trait, and

removing said contaminating transgenic immunodeficient recipient cells from said culture, wherein said step of removing results in a substantially purified population of cells.

2. The method of claim 1 wherein said transgenic immunodeficient recipient organism is a mouse.

3. The method of claim 1 wherein said donor cells are selected from the group consisting of tumor cells, pancreatic islet cells, lymphocytes and hematopoietic bone marrow cells.

4. The method of claim 1 wherein said selectable trait is selected from the group consisting of ability to express a toxic protein, ability to express a detectable marker, a biochemical deficiency, and sensitivity to a selection agent.

5. The method of claim 4 wherein said toxic protein is thymidine kinase.

6. The method of claim 1 wherein said step of selecting is carried out by exposing said culture to an effector selected from the group consisting of a selection agent and an environmental stimulus.

7. The method of claim 6 wherein said selection agent is gangcyclovir.

8. The method of claim 6 wherein said environmental stimulus is selected from the group consisting of light, temperature change, and withdrawal of an essential nutrient.

9. The method of claim 1 wherein said step of removing is carried out by mechanical removal of said contaminating transgenic immunodeficient recipient cells.

10. The method of claim 1 wherein said step of removing is carried out by inducing the death of said contaminating transgenic immunodeficient recipient cells.

11. The method of claim 1 further comprising the step of activating an inducible promoter that controls transcription of a gene encoding said selectable trait.

12. The method of claim 11 wherein said inducible promoter is selected from the group consisting of tetracycline-inducible promoter, lac-inducible promoter, and metallothionein promoter.

13. The method of claim 1, further comprising the step of establishing a cell line from said substantially purified population of cells.

14. A substantially purified population of cells, wherein said cells are substantially purified by

obtaining donor cells from a donor organism,

implanting said donor cells into a non-human transgenic immunodeficient recipient organism, wherein cells of said transgenic immunodeficient recipient organism exhibit a selectable trait,

harvesting said donor cells from said transgenic immunodeficient recipient organism,

placing said donor cells in culture,

selecting contaminating transgenic immunodeficient recipient cells in said culture via

said selectable trait, and

removing said contaminating transgenic immunodeficient recipient cells from said culture, wherein said step of removing results in a substantially purified population of cells.

15. The population of cells of claim 14 wherein said cells are selected from the group consisting of tumor cells, pancreatic islet cells, lymphocytes and hematopoietic bone marrow cells.

16. A vaccine comprising

a substantially purified population of cells wherein said cells are substantially purified by

obtaining donor cells from a donor organism,

implanting said donor cells into a non-human transgenic immunodeficient recipient organism, wherein cells of said transgenic immunodeficient recipient organism exhibit a selectable trait,

harvesting said donor cells from said transgenic immunodeficient recipient organism,

placing said donor cells in culture,

selecting contaminating transgenic immunodeficient recipient cells in said culture via said selectable trait, and

removing said contaminating transgenic immunodeficient recipient cells from said culture, wherein said step of removing results in a substantially purified population of cells, and

a physiological acceptable carrier.

17. The vaccine of claim16 further comprising an adjuvant.

18. A non-human, immunodeficient animal

wherein cells of said animal are transgenic for a selectable trait.

19. The animal of claim 18, wherein said animal is a mouse.

20. The animal of claim 19, wherein said mouse is an offspring of a nu/nu mouse.

21. The animal of claim 19, wherein said animal is a tox/tox:nu/nu mouse.

22. The animal of claim 18, wherein said selectable trait is selected from the group consisting of ability to express a toxic protein, ability to express a detectable marker, a biochemical deficiency, and sensitivity to a selection agent.

23. The animal of claim 22 wherein said toxic protein is thymidine kinase.

24. The animal of claim 22, wherein the said detectable marker is selected from the group consisting of green fluorescent protein, luciferase, expression of a cell surface protein and expression of a cell surface polysaccharide.

25. The animal of claim 22, wherein said biochemical deficiency is selected from the group consisting of ARIFT deficiency and HPRT deficiency.

26. A method for testing the effect of a compound on cells of a selected type, comprising the steps of

procuring a population of substantially purified cells of said selected type by

obtaining donor cells from a donor organism,

implanting said donor cells into a non-human transgenic immunodeficient recipient organism, wherein cells of said transgenic immunodeficient recipient organism exhibit a selectable trait,

harvesting said donor cells from said transgenic immunodeficient recipient organism,

placing said donor cells in culture,

selecting contaminating transgenic immunodeficient recipient cells in said culture via said selectable trait, and

removing said contaminating transgenic immunodeficient recipient cells from said culture, wherein said step of removing results in a substantially purified population of cells

exposing said substantially purified population of cells to said compound, and

observing the effect of said compound on said substantially purified population of cells.

27. The method of claim 26, wherein said compound is selected from the group consisting of a drug, a toxin, an anti-tumor agent, libraries of chemicals, extracts, peptides, and polysaccharides.

28. The method of claim 26 wherein said cells of a selected type are tumor cells and said compound is an anti-tumor compound.

29. A cell line, comprising,

a substantially purified population of cells, wherein said cells are substantially purified by

obtaining donor cells from a donor organism,

implanting said donor cells into a non-human transgenic immunodeficient recipient organism, wherein cells of said transgenic immunodeficient recipient organism exhibit a selectable trait,

harvesting said donor cells from said transgenic immunodeficient recipient organism,

placing said donor cells in culture,

selecting contaminating transgenic immunodeficient recipient cells in said culture via said selectable trait, and

removing said contaminating transgenic immunodeficient recipient cells from said culture, wherein said step of removing results in a substantially purified population of cells.

30. The cell line of claim 29, wherein said cell line is immortal.

31. The cell line of claim 29, wherein said cells are tumor cells.

32. A kit, comprising,

a substantially purified population of cells, wherein said cells are substantially purified by

obtaining donor cells from a donor organism,

implanting said donor cells into a non-human transgenic immunodeficient recipient organism, wherein cells of said transgenic immunodeficient recipient organism exhibit a selectable trait,

harvesting said donor cells from said transgenic immunodeficient recipient organism,

placing said donor cells in culture,

selecting contaminating transgenic immunodeficient recipient cells in said culture via said selectable trait, and

removing said contaminating transgenic immunodeficient recipient cells from said culture, wherein said step of removing results in a substantially purified population of cells;

medium; and

a container to contain said cells and said medium.

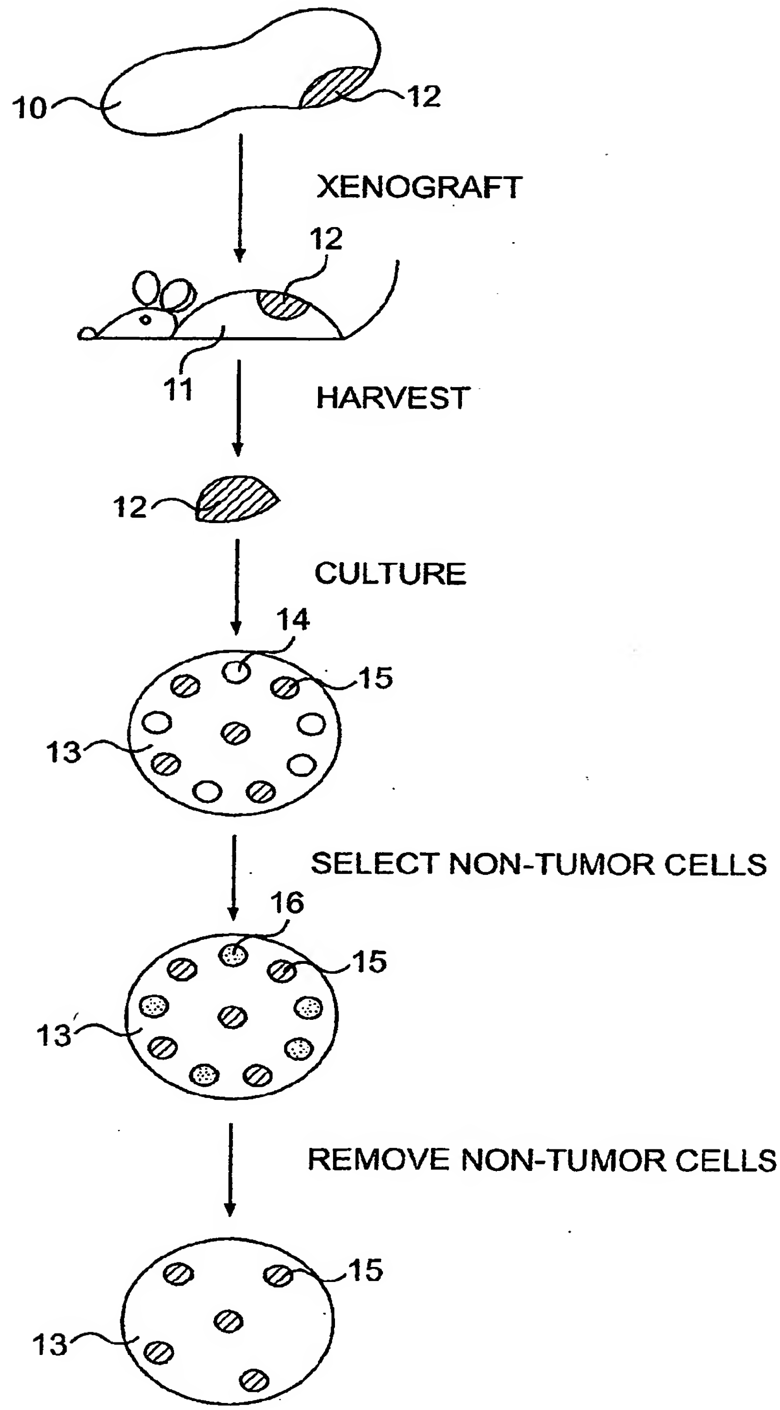
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FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/31219

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL : 424/93.2; 435/325; 800/8, 13, 21, 24, 25
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.2; 435/325; 800/8, 13, 21, 24, 25

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHALFIE ET AL. Green Fluorescent Protein as a Marker for Gene Expression. Science. 11 FEBRUARY 1994, Vol. 263, pages 802-805.	1-32
A	EDAMURA ET AL. No Self-Injurious Behavior was Found in HPRT-Deficient Mice Treated With 9-Ethyladenine. Pharmacology Biochemistry and Behavior. 1998, Vol. 61, No. 2, pages 175-179.	18-25
Y -- A	STOCKELMAN ET AL. Chronic Renal Failure in a Mouse Model of Human Adenine Phosphoribosyltransferase Deficiency. American Journal of Physiology. 1998, Vol. 275, No. 44, pages F154-F163.	18-19 ------ 20-25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"O"	document referring to an oral disclosure, use, exhibition or other means		
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Date of the actual completion of the international search	Date of mailing of the international search report
11 DECEMBER 2001	13 FEB 2002

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/31219

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOREADITH ET AL. Gene Targeting in Embryonic Stem Cells. The New Physiology and Metabolism. Journal of Molecular Medicine. 1997, Vol. 75, pages 208-216.	1-32
A	POLEJAEVA ET AL. New Advances in Somatic Cell Nuclear Transfer Application in Transgenesis. Theriogenology. 2000, Vol. 53, pages 117-126.	1-32
Y	Immunodeficient Rodents Opening New Doors for Investigators. March 1996, Vol. 1, No. 2, pages 1-3. [online], [retrieved on 2001-12-12] Retrieved from the Internet <URL:http://www.taconic.com/newsltrs/march96/march96a.htm>	18-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/31219

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

A01N 63/00; A61K 48/00; C12N 5/00, 5/02, 15/00; A01K 67/00, 67/033

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

STN, WEST 2.0
search terms: scott kern, non-human immunodeficient animal, transgenic mouse, selectable trait, selectable marker, cells, purified population of cells, vaccine

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